REF 300900 NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip

℞ only

CAUTION: For US Export Only

IVD For *in vitro* diagnostic use with the NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems

For insert updates, go to: www.qiagen.com/neumodx-ifu
For detailed instructions, refer to the NeuMoDx™ 288 Molecular System Operator's Manual; P/N 40600108
For detailed instructions, refer to the NeuMoDx™ 96 Molecular System Operator's Manual; P/N 40600317

INTENDED USE

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay performed on the NeuMoDx™ 288 Molecular System and NeuMoDx™ 96 Molecular System (NeuMoDx Molecular System(s)), is a multiplex, rapid, automated, qualitative in vitro real-time RT-PCR diagnostic test intended for simultaneous direct detection and differentiation of influenza A virus , influenza B virus, respiratory syncytial virus (RSV) and SARS-CoV-2 RNA from nasopharyngeal (NP) swabs in transport medium from individuals with signs and symptoms of respiratory tract infection in conjunction with clinical and epidemiological risk factors.

The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of active infection. Negative results do not preclude influenza virus, RSV, or SARS-Cov-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A and B virus detection were established with clinical specimens collected during the 2019/2020 influenza season. When other influenza A and B viruses are emerging, performance characteristics may vary.

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures and/or NeuMoDx Molecular Systems.

SUMMARY AND EXPLANATION

Nasopharyngeal swab specimens are collected in Copan Universal Transport Medium (UTM-RT®) System, BD™ Universal Viral Transport System (UVT), or Biologos Bio-VTM™ Viral Transport Media (VTM). The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay allows for two specimen processing workflows based on laboratories' need. To prepare for testing using the Direct Workflow, the primary collection tube (with swab and cap removed) or an aliquot of the sample medium in a secondary tube is barcoded and loaded onto the NeuMoDx System using a designated specimen tube carrier. For the Pretreated Workflow, the specimen in transport medium is first treated with equal volume of NeuMoDx Vantage Viral Lysis Buffer (VVLB) before it is loaded onto the System. For the Direct Workflow, a 400 µL aliquot of the sample is aspirated by the NeuMoDx System and mixed with an equal volume of NeuMoDx Lysis Buffer 3 while for the Pretreated Workflow 550µL of the pretreated sample is combined with an equal volume of Lysis Buffer 2. The NeuMoDx Molecular System automatically performs all the steps required to extract the target nucleic acid, prepare the isolated RNA for real-time reverse transcriptase polymerase chain reaction (RT-PCR) and, if present, amplify and detect the products of amplification. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay targets the conserved region of SARS-CoV-2 Nsp2 gene and regions in the M genes of influenza A, influenza B and respiratory syncytial virus A or B genomes. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay includes an RNA Sample Process Control (SPC2) to help monitor for the presence of potentially inhibitory substances and NeuMoDx System or reagent failures that may be encountered during the extraction and amplification process.

PRINCIPLES OF THE PROCEDURE

The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Assay combines automated RNA extraction and amplification/detection by real-time RT-PCR. Nasopharyngeal swab samples are collected in the Copan UTM-RT® System, BD™ UVT System, or Biologos Bio-VTM™ Viral Transport Media (VTM). The Direct Workflow allows the primary swab collection tube or an aliquot of the transport medium in a secondary tube to be barcoded and loaded onto the NeuMoDx System for processing. Alternatively, an NP swab specimen in transport medium can be first treated with equal volume of NeuMoDx Vantage Viral Lysis Buffer (VVLB) before loaded onto the System without further user intervention. The NeuMoDx System automatically aspirates either an aliquot of specimens to mix with NeuMoDx Lysis Buffer 3 for direct workflow or an aliquot of pretreated specimen to mix with Lysis Buffer 2 and the reagents contained in the NeuMoDx™ Extraction Plate to begin processing. The NeuMoDx System automates and integrates RNA extraction and concentration, reagent preparation, and nucleic acid amplification/detection of the target sequences using real-time RT-PCR. The included Sample Process Control (SPC2) helps monitor for the presence of inhibitory substances and for system, process, or reagent failures. No operator intervention is necessary once the specimen is loaded onto the NeuMoDx System.

The NeuMoDx System uses a combination of heat, lytic enzyme, and extraction reagents to automatically perform lysis, RNA extraction, and removal of inhibitors. The released nucleic acids are captured by paramagnetic particles. The particles, with bound nucleic acid, are loaded into the NeuMoDx™ Cartridge where the unbound elements are washed away with NeuMoDx™ Wash Reagent. The bound RNA is then eluted using NeuMoDx™ Release Reagent. The NeuMoDx System uses the eluted RNA to rehydrate proprietary NeuDry™ amplification reagents containing all the elements necessary for amplification of the Flu A, Flu B, RSV, SARS-CoV-2 and SPC2 targets. This enables simultaneous amplification and detection of all targets and sample process control RNA sequences. Upon reconstitution of the dried RT-PCR reagents, the NeuMoDx System dispenses the prepared RT-PCR-ready mixture into one PCR chamber (per specimen) of the NeuMoDx Cartridge. Reverse transcription, amplification, and detection of the control and target sequences (if present) occur in the PCR chamber. The NeuMoDx Cartridge is designed to contain the generated amplicon following RT-PCR, virtually eliminating the risk of post-amplification contamination.

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons of their respective targets. TaqMan probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe is intact, the fluorophore and the quencher are in proximity, allowing the quencher molecule to suppress the fluorescence emitted by the fluorophore via Förster Resonance Energy Transfer (FRET).

TaqMan® probes are designed such that they anneal within a DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore and breaks its proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing detection of the fluorophore. The resulting fluorescent signal detected in the NeuMoDx System quantitative RT-PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target present.

TaqMan® probes are labeled with fluorophores at the 5' end and a dark quencher at the 3' end and are utilized to detect the viral targets. The fluorescent detection channel for each NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay target is presented in *Table 1*. The NeuMoDx System software monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When thermal cycling is complete, the NeuMoDx System software analyzes the data and reports a result (POSITIVE/NEGATIVE/INDETERMINATE/NO RESULT/UNRESOLVED).

Table 1. Detection Channel

Organism	Target Region	Probe Fluorophore	Excitation/Emission	Detection Channel
Influenza A	M gene	HEX	530/555 nm	Yellow
Influenza B	M gene	FAM	470/510 nm	Green
SARS-CoV-2	Nsp2 gene	Texas Red	585/610 nm	Orange
Respiratory Syncytial Virus	M gene	Q705	680/715 nm	Far Red
SPC2	Assembly Protein (MS2)	Q670	625/660 nm	Red

REAGENTS / CONSUMABLES

Material Provided

REF	Contents	Units per package	Tests per unit	Tests per package
300900	**NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip** *Dried RT-PCR reagents containing Flu A-B/RSV/SARS-CoV-2 specific TaqMan® probes and primers, and SPC2 specific TaqMan® probe and primers.* *Contains 21.1% Tris-HCl, 8.4% dNTP and other inactive ingredients*	6	16	96

Materials Required but Not Provided (Available Separately from NeuMoDx)

REF	Contents
100200	**NeuMoDx™ Extraction Plate** *Dried paramagnetic particles, lytic enzyme, and sample process controls*
400500**	**NeuMoDx™ Lysis Buffer 2**
400600*	**NeuMoDx™ Lysis Buffer 3**
401500**	**NeuMoDx™ Vantage Viral Lysis Buffer**
400100	**NeuMoDx™ Wash Reagent**
400200	**NeuMoDx™ Release Reagent**
100100	**NeuMoDx™ Cartridge**
235903	**Hamilton® CO-RE Tips (300 µL) with Filters**
235905	**Hamilton® CO-RE Tips (1000 µL) with Filters**

** Required only for direct processing of samples, without a pretreatment step. See "Instructions for Use" section below.*
*** Required only if a pretreatment step is desired prior to loading samples. See "Instructions for Use" section below.*

Swabs and Transport Media (Not Provided)

Sample Type	Recommended Collection Device	Recommended Swab
Nasopharyngeal Swab	3 mL Universal Transport Medium (Copan UTM-RT®, Copan, CA, USA) **or** 3 mL Universal Viral Transport System (BD™ UVT, BD, NJ, USA) **or** 3mL Bio-VTM™ Viral Transport Medium (Bio-VTM™, Biologos LLC, IL, USA)	Flexible Minitip Nylon® Flocked Swab (Copan, CA, USA) **or** Flexible Minitip Flocked Swab (BD, NJ, USA)

Instrumentation Required

NeuMoDx™ 288 Molecular System [REF 500100] or **NeuMoDx™ 96 Molecular System** [REF 500200]

WARNINGS AND PRECAUTIONS

- The NeuMoDx™ Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip is for *in vitro* diagnostic use with NeuMoDx™ Systems only.
- Do not use the reagents or consumables after the listed expiration date.
- Do not use any reagents if the safety seal is broken or if the packaging is damaged upon arrival.
- Do not use consumables or reagents if the protective pouch is open or broken upon arrival.
- Minimum specimen volume of secondary aliquots is dependent on the tube size/specimen tube carrier as defined below. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
- The use of specimens stored at improper temperatures or beyond the specified storage times may produce invalid or erroneous results.
- Avoid microbial and ribonuclease (RNase) contamination of all reagents and consumables. The use of sterile, DNase-free, disposable transfer pipettes is recommended when using secondary tubes. Use a new pipette for each specimen.
- To avoid contamination, do not handle or break apart any NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from the Biohazard Waste Container (NeuMoDx 288 Molecular System) or Biohazard Waste Bin (NeuMoDx 96 Molecular System) under any circumstances. The NeuMoDx Cartridge is designed to prevent contamination.
- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of the NeuMoDx Cartridge, the foil seal surface of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip and the NeuMoDx Extraction Plate, or the top surface of the NeuMoDx Lysis Buffer container; handling of the consumables and reagents should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are provided for each reagent (as applicable) at *www.qiagen.com/safety*.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[1] and in CLSI Document M29-A4.[2]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state, and local regulations.
- Do not reuse.

PRODUCT STORAGE, HANDLING AND STABILITY

- NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strips are stable in the primary packaging through the stated expiration date on the immediate product label when stored at 4°C to 28°C.
- Do not use consumables and reagents past the stated expiration date.
- Do not use any test product if the primary or secondary packaging has been visually compromised.
- Do not reload any test product that has previously been loaded onto another NeuMoDx System.
- Once loaded, the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip may remain onboard the NeuMoDx System for 7 days. Remaining shelf life of loaded test strips is tracked by the software and reported to the user in real time. Removal of a test strip that has been in use beyond its allowable period will be prompted by the System.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Handle all specimens as if they are capable of transmitting infectious agents.

1. Specimens should be collected using the Copan UTM-RT® System, BD™ UVT System or Bio-VTM™ using the validated nylon flocked swabs (see Swabs and Transport Media). In addition, flocked swabs, polyester, and rayon swabs are acceptable swab types. Follow manufacturer instructions for specimen collection, transport, and storage.
2. Specimens may be tested in primary collection tubes or secondary specimen tubes.
3. Specimen tubes may be stored on the NeuMoDx System for up to 8 hours prior to processing. If additional storage time is required, it is recommended that the specimens be either refrigerated or frozen as secondary aliquots.
4. Prepared specimens should be stored at 2 to 8 °C for no longer than 7 days prior to testing.
5. If specimens are shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations.
6. Proceed to *Test Preparation* section.

INSTRUCTIONS FOR USE

The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay accommodates two different workflows, depending on user/laboratory preference:

Workflow 1: **DIRECT** – swab specimen in transport medium is loaded directly onto the NeuMoDx System in primary collection tube or secondary specimen tubes

-or-

Workflow 2: **PRETREATED** – swab specimen in transport medium is pretreated with NeuMoDx Vantage Viral Lysis Buffer before it is loaded onto the NeuMoDx System in primary collection tube or secondary specimen tubes

Test Preparation – DIRECT Workflow for Direct Swab Samples

1. Apply specimen barcode label to a specimen tube compatible with the NeuMoDx System as described under step 4 below.
2. If testing the specimen in the primary collection tube, place the barcoded tube into a Specimen Tube Carrier and ensure the cap and swab are removed prior to loading onto the NeuMoDx System.
3. Alternatively, an aliquot of the transport medium may be transferred to a barcoded secondary tube and placed into a Specimen Tube Carrier. If using a secondary tube, transfer an aliquot of the transport medium to the barcoded specimen tube compatible with the NeuMoDx System according to the volumes defined below:
4. *For swab specimens:*
   - Specimen Tuber Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 550 µL
   - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 1000 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 500 µL

Test Preparation – PRETREATED Workflow for Pretreated Swab Samples

Note: Bring Vantage Viral Lysis Buffer to room temperature (15 to 30 °C) before using.

WARNING: Pretreatment of swab samples with NeuMoDx Vantage Viral Lysis Buffer does not guarantee inactivation of any virus present. All samples should be handled as if they are capable of transmitting infectious agents.

1. Pretreat the sample transport medium with a 1:1 volume of NeuMoDx Vantage Viral Lysis Buffer. This can be done in the primary swab collection tube if the volume of transport medium is known. Alternatively, pretreatment can be done in a secondary tube by combining an aliquot of the transport medium with an equal volume of NeuMoDx Vantage Viral Lysis Buffer. The resulting mixture should meet the minimum volume requirements specified below.
2. Mix gently with a pipette to ensure uniform distribution of NeuMoDx Vantage Viral Lysis Buffer.
3. If testing the pretreated specimen in the primary collection tube, place the barcoded tube into a Specimen Tube Carrier and ensure the cap and swab are removed prior to loading onto the NeuMoDx System.
4. If using a secondary tube, transfer an aliquot of the pretreated sample to a barcoded specimen tube compatible with the NeuMoDx System and place into a Specimen Tube Carrier according to the volumes defined below:
   - Specimen Tube Carrier (32-tube): 11 – 14 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 700 µL
   - Specimen Tube Carrier (24-tube): 14.5 – 18 mm in diameter and 60 – 120 mm in height; minimum fill volume ≥ 1100 µL
   - Low Volume Specimen Tube Carrier (32-tube): 1.5 mL conical bottom microcentrifuge tube; minimum fill volume ≥ 650 µL

NeuMoDx System Operation

For detailed instructions, refer to the NeuMoDx™ 288 and 96 Molecular Systems Operator's Manuals (P/N 40600108 & 40600317)

1. Load the test order onto the NeuMoDx System according to the workflow used for test preparation:
   - Untreated, neat swab samples prepared using the DIRECT workflow are tested by defining the sample as "**Transport Medium**"
   - Swab samples pretreated with VVLB using the PRETREATED workflow are tested by defining the specimen as "**UserSpecified1**"
2. Populate one or more NeuMoDx™ System Test Strip Carrier(s) with NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip(s) and use the touchscreen to load the Test Strip Carrier(s) into the NeuMoDx System.
3. If prompted by the NeuMoDx™ System software, add the necessary required consumables to the NeuMoDx System consumable carriers and use the touchscreen to load carrier(s) into the NeuMoDx System.
4. If prompted by the NeuMoDx System software, replace NeuMoDx Wash Reagent, NeuMoDx Release Reagent, empty the Priming Waste, Biohazard Waste Container (NeuMoDx 288 Molecular System only), Tip Waste Bin (NeuMoDx 96 Molecular System only), or Biohazard Waste Bin (NeuMoDx 96 Molecular System only), as appropriate.
5. Load the specimen tube(s) into a Specimen Tube Carrier and ensure caps and any swabs are removed from all tubes.
6. Place the Specimen Tube Carrier(s) on the autoloader shelf and use the touchscreen to load the carrier(s) into the NeuMoDx System. This will initiate processing of the loaded specimens for the tests identified, given a valid test order is present in the system.

LIMITATIONS

1. The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip can only be used on NeuMoDx Systems.
2. The performance of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip has been established for clinician-collected nasopharyngeal swab samples in transport medium. Use of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip with other sources has not been assessed and performance characteristics are unknown for other specimen types.
3. Because detection of viral targets is generally dependent on the number of viral particles present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
4. Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube mix-up. In addition, false negative results could occur because the number of viral particles in the sample is below the limit of detection of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay.
5. Operation of the NeuMoDx System is limited to use by personnel trained on the use of the NeuMoDx System.
6. If Flu A, B, RSV and SARS-CoV-2 targets and the SPC2 target do not amplify, an invalid result (Indeterminate or Unresolved) will be reported and the test should be repeated.
7. If a system error occurs prior to completion of sample processing, "No Result" will be reported and the test should be repeated.
8. A positive result does not necessarily indicate the presence of viable influenza A, influenza B, SARS-CoV-2 and/or respiratory syncytial virus. However, a positive result is presumptive for the presence of influenza A, influenza B, SARS-CoV-2 and/or respiratory syncytial virus (A or B) RNA.
9. The NeuMoDx™ Flu A-B/RSV/SARS-CoV2 Vantage Test Strip may contain inactive ingredients that may influence the measurement.
10. Deletions or mutations in the conserved regions targeted by the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay may affect detection and could lead to an erroneous result.
11. Results from NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay should be used as an adjunct to clinical observations and other information available to the physician.
12. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination.

RESULTS

Available results may be viewed or printed from the 'Results' tab in the Results window on the NeuMoDx System touchscreen. NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay results are automatically generated by the NeuMoDx System software using the decision algorithm and results processing parameters specified in the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay Definition File (Flu A-B-RSV SARS-CoV-2 ADF version 4.0.0 or higher). A NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay result may be reported as Negative, Positive, Indeterminate, No Result, or Unresolved based on the amplification status of the target and sample process control. Results are reported based on the ADF results processing decision algorithm, summarized below in *Table 2*.

Table 2. NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay Results Interpretation

RESULT	Flu A Target	Flu B Target	RSV Target	SARS-CoV-2 Target	PROCESS CONTROL (SPC2)	Interpretation
POSITIVE	**Amplified**	N/A	N/A	N/A	N/A	Flu A RNA Detected
	N/A	**Amplified**	N/A	N/A	N/A	Flu B RNA Detected
	N/A	N/A	**Amplified**	N/A	N/A	RSV RNA Detected
	N/A	N/A	N/A	**Amplified**	N/A	SARS-CoV-2 RNA Detected
NEGATIVE	**Not Amplified**	**Not Amplified**	**Not Amplified**	**Not Amplified**	**Amplified**	Flu A, Flu B, RSV, and SARS-CoV-2 RNA not detected
NO RESULT*	Not Amplified, System Error Detected, Sample Processing Aborted					All target results were invalid; retest sample
IND*	Not Amplified, System Error Detected, Sample Processing Completed					Sample processing was aborted; retest sample
UNR*	Not Amplified, No System Error Detected					All target results were invalid; retest sample

* The System allows optional Rerun/Repeat capability to enable automatic reprocessing in the event of an invalid result to minimize delays in result reporting.

Invalid Results

If a NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay performed on the NeuMoDx System fails to produce a valid result, it will be reported as either Indeterminate, No Result, or Unresolved based on the type of error that occurred, and the test should be repeated to obtain a valid result.

An Indeterminate result will be reported if a NeuMoDx System error is detected during sample processing. In the event of an Indeterminate result, a retest is recommended.

A No Result will be reported if a NeuMoDx System error is detected and sample processing is aborted. In the event of a No Result, a retest is recommended.

An Unresolved result will be reported if no target is detected and there is no amplification of the Sample Process Control, which indicates possible reagent failure or the presence of inhibitors. In the event of an Unresolved result, a retest is recommended as a first step. If the retest fails, a diluted specimen may be used to mitigate the effect of possible inhibition.

See the NeuMoDx 288 Molecular System Operator's Manual (PN: 40600108) or the NeuMoDx 96 Molecular System Operator's User Manual (PN: 40600317) for a list of error codes that may be associated with any Invalid Results.

Quality Control

Local regulations typically specify that the laboratory is responsible for control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials.

Control Materials will not be provided by NeuMoDx Molecular, Inc. Appropriate controls must be chosen and validated by the laboratory. Note that the controls must meet the same minimum volume specifications as clinical samples specified above based on the Specimen Tube Carrier size. The following materials are recommended as control material:

- Positive Control (1 mL per control):
  - 5 µL RSV Rapid Control Pack (Zeptometrix, Cat #: KZMC034)
  - 5 µL NATrol Influenza A/B Positive Control (Zeptometrix, Cat #: MDZ046)
  - Heat-inactivated SARS-CoV-2 virus (ATCC, VR-1986HK) at a final concentration of 1000 cp/mL
  - BD™ Universal Viral Transport Medium (UVT) or equivalent to final volume of 1 mL

- Negative Control: BD™ Universal Viral Transport Medium (UVT, BD, NJ) or equivalent

When processing User-Defined Controls, place the labeled controls in a specimen tube carrier and use the touchscreen to load the carrier into the NeuMoDx System from the autoloader shelf. Once defined (See NeuMoDx 288 Molecular System Operator's Manual (P/N: 40600108) or the NeuMoDx 96 Molecular System Operator's User Manual (P/N: 40600317)), the NeuMoDx System will recognize the associated barcodes and automatically start processing them as controls.

It is recommended that users process one set of positive and negative controls prior to processing patient samples, once every 24 hours of System operation.

Sample Process (Internal) Controls

An exogenous Sample Process Control (SPC2) is incorporated in the NeuMoDx Extraction Plate and undergoes the entire process of nucleic acid extraction and real-time RT-PCR amplification with each sample. Primers and probe specific to SPC2 are also included in each NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip well, enabling detection of SPC2 with the target RNA (if present) via multiplex PCR. Detection of SPC2 amplification allows the NeuMoDx System software to monitor the efficacy of the RNA extraction and PCR amplification processes.

Prior to RT-PCR, the NeuMoDx System automatically performs a 'FILL CHECK' to ensure that the PCR chamber is filled with solution and contains an adequate amount of fluorescent probe.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The Analytical Sensitivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay on the NeuMoDx Molecular Systems was characterized in two parts. First, a dilution series using model strains of each target in UVT were prepared with the Pretreated Workflow and then processed by the NeuMoDx System to determine a preliminary Limit of Detection (LoD) value. In the second part of testing, this preliminary LoD value was confirmed using a hit-rate study on both the NeuMoDx 288 and the NeuMoDx 96 Molecular Systems for both workflows. The preliminary LoD was accepted if the hit-rate testing achieved a 95% positivity rate for both workflows on both Systems. Detection rates for the preliminary LoD are depicted in *Table 3* while *Table 4* details the hit-rate confirmation for the N288 System and Table 5 details the hit-rate confirmation for the N96 System.

Table 3. Positive Detection Rates for Preliminary LoD Determination of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay

Target/Strain	Level	Unit	# Valid Results	# Positives	% Detection
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5	$TCID_{50}$/mL	10	10	100%
	0.25		10	9	90.0%
Flu A, Michigan/272/2017 pdm09 (H1N1)	0.5		10	10	100%
	0.25		10	8	80.0%
Flu B, Colorado/6/2017 (Victoria)	0.25		10	10	100%
	0.05		10	10	100%
	0.01		8	8	100%
Flu B, Florida/78/2015 (Yamagata)	0.25		10	10	100%
	0.1		10	9	90.0%
RSV A2	0.5		9	9	100%
	0.25		9	8	88.9%
RSV B (WV/14617/85)	0.25		10	10	100%
	0.05		9	9	100%
SARS-CoV-2, Isolate USA-WA1/2020	300	copies/mL	10	10	100%
	200		10	10	100%
	150		10	10	100%
	100		10	7	70.0%

Table 4. Positive Detection Rates for Hit-Rate Confirmation of LoD for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay – N288, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	# Valid Results	# Positives	% Detection
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5 $TCID_{50}$/mL	24	24	100%
Flu A, Michigan/272/2017 pdm09 (H1N1)	0.5 $TCID_{50}$/mL	23	23	100%
Flu B, Colorado/6/2017 (Victoria)	0.01 $TCID_{50}$/mL	24	24	100%
Flu B, Florida/78/2015 (Yamagata)	0.25 $TCID_{50}$/mL	24	24	100%
RSV A2	0.5 $TCID_{50}$/mL	21	20	95.2%
RSV B (WV/14617/85)	0.25 $TCID_{50}$/mL	22	22	100%
SARS-CoV-2, Isolate USA-WA1/2020	150 copies/mL	23	23	100%
SARS-CoV-2, Isolate Italy-INMI1	150 copies/mL	23	23	100%

(b) Direct Workflow

Target/Strain	Level	# Valid Results	# Positives	% Detection
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5 $TCID_{50}$/mL	24	24	100%
Flu A, Michigan/272/2017 pdm09 (H1N1)	0.5 $TCID_{50}$/mL	24	24	100%
Flu B, Colorado/6/2017 (Victoria)	0.01 $TCID_{50}$/mL	24	23	95.8%
Flu B, Florida/78/2015 (Yamagata)	0.25 $TCID_{50}$/mL	24	24	100%
RSV A2	1 $TCID_{50}$/mL	24	24	100%
RSV B (WV/14617/85)	0.05 $TCID_{50}$/mL	24	24	100%
SARS-CoV-2, Isolate USA-WA1/2020	250 copies/mL	23	22	95.7%
SARS-CoV-2, Isolate Italy-INMI1	250 copies/mL	23	23	100%

Table 5. Positive Detection Rates for Hit-Rate Confirmation of LoD for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay – N96, (a) Pretreated Workflow; (b) Direct Workflow

(a) Pretreated Workflow

Target/Strain	Level	# Valid Results	# Positives	% Detection
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5 $TCID_{50}$/mL	24	23	95.8%
Flu A, Michigan/272/2017 pdm09 (H1N1)	0.5 $TCID_{50}$/mL	22	21	95.5%
Flu B, Colorado/6/2017 (Victoria)	0.01 $TCID_{50}$/mL	24	23	95.8%
Flu B, Florida/78/2015 (Yamagata)	0.25 $TCID_{50}$/mL	24	24	100%
RSV A2	0.5 $TCID_{50}$/mL	22	22	100%
RSV B (WV/14617/85)	0.25 $TCID_{50}$/mL	23	23	100%
SARS-CoV-2, Isolate USA-WA1/2020	150 copies/mL	23	22	95.7%
SARS-CoV-2, Isolate Italy-INMI1	150 copies/mL	22	21	95.5%

(b) Direct Workflow

Target/Strain	Level	# Valid results	# POS	% Detection
Flu A, Singapore/INIFMIH-16-0019/2016 (H3N2)	0.5 $TCID_{50}$/mL	24	23	95.8%
Flu A, Michigan/272/2017 pdm09 (H1N1)	0.5 $TCID_{50}$/mL	23	23	100%
Flu B, Colorado/6/2017 (Victoria)	0.01 $TCID_{50}$/mL	24	24	100%
Flu B, Florida/78/2015 (Yamagata)	0.25 $TCID_{50}$/mL	24	23	95.8%
RSV A2	1 $TCID_{50}$/mL	22	22	100%
RSV B (WV/14617/85)	0.05 $TCID_{50}$/mL	23	23	100%
SARS-CoV-2, Isolate USA-WA1/2020	250 copies/mL	23	22	95.7%
SARS-CoV-2, Isolate Italy-INMI1	250 copies/mL	23	22	95.7%

The levels accepted as the LoD values for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay on the NeuMoDx Systems are summarized in *Table 6.* The Limit of Detection of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay is claimed to be 0.5 $TCID_{50}$/mL for Flu A, 0.25 $TCID_{50}$/mL for Flu B, 1.0 $TCID_{50}$/mL for RSV A, 0.05 $TCID_{50}$/mL for RSV B and 250 copies/mL for SARS-CoV-2.

Table 6. Summary of Limit of Detection Study

Target	Strain	Limit of Detection		
		Pretreated Workflow	Direct Workflow	Unit
Influenza A (Flu A) – H3N2	Singapore/INIFMIH-16-0019/2016	0.5	0.5	$TCID_{50}$/mL
Influenza A (Flu A) – H1N1	Michigan/272/2017 pdm09	0.5	0.5	
Influenza B (Flu B) – Victoria lineage	Colorado/6/2017	0.01	0.01	
Influenza B (Flu B) – Yamagata lineage	Florida/78/2015	0.25	0.25	
RSV A	A2	0.25	1	
RSV B	(WV/14617/85)	0.05	0.05	
SARS-CoV-2	Isolate USA-WA1/2020	150	250	copies/mL

Competitive Interference on SARS-CoV-2 Detection

Analytical sensitivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was evaluated in the context of a contrived co-infection of SARS-CoV-2 with the other three targets, Flu A, Flu B, or RSV. This scenario was evaluated with samples prepared by diluting heat-inactivated SARS-CoV-2 with pre-screened negative swab matrix to 1X LoD in the presence of Flu A, Flu B, and/or RSV targets at concentrations ≥ 3 Log_{10} $TCID_{50}$/mL of their respective LoD levels. The detection rate of SARS-CoV-2 at LoD level was not impacted adversely by the presence of high viral titer of Flu A, Flu B, RSV A, or RSV B, *Table 7*.

Table 7. Summary of Competitive Interference Study

		SARS-CoV-2			Flu A, Flu B, RSV A or RSV B		
Sample	**n**	**% Positive**	**Ave Ct**	**SD**	**% Positive**	**Ave Ct**	**SD**
SARS-CoV-2 /Flu A	24	96%	33.53	0.42	100%	25.22	0.53
SARS-CoV-2 /Flu B	24	96%	34.01	0.72	100%	24.43	0.46
SARS-CoV-2 /RSV A	24	100%	33.76	0.44	100%	19.47	0.69
SARS-CoV-2 /RSV B	24	100%	33.84	0.43	100%	20.55	0.62

Analytical Reactivity and Inclusivity

The reactivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was evaluated against multiple strains/isolates of Influenza A, Influenza B, Respiratory Syncytial Viruses and SARS-CoV-2. Viral strains/isolates were tested in minimum 20 replicates. A total of 24 Flu A strains, 6 Flu B strains, 3 RSV A isolates, 2 RSV B isolates, and 4 isolates of SARS-CoV-2 were tested, *Table 8*.

Table 8. Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 Strains Tested

Target	Strain		Concentration	% Pos
Flu A	H1N1	Brisbane/02/2018	1 $TCID_{50}$/mL	95.5%
		California/07/2009	1 $TCID_{50}$/mL	100%
		California/07/2009 NYMC X-179A (H1N1)pdm09	18 $TCID_{50}$/mL	95.5%
		Louisiana/08/2013 pdm 09, AVR Reference Strain, M2: S31N, NA: H275Y	8 $TCID_{50}$/mL	100%
		New York/18/2009 (H1N1)pdm09	6 $TCID_{50}$/mL	100%
		Guangdong-Moanan/SWL 1536/2019	1 $TCID_{50}$/mL	100%
	H2N2	A2/Japan/305/57	32.6 pg/mL	100%
		Korea/426/68 (HA, NA) x A/PR/8/34	6.25 pg/mL	100%
	H3N2	Hong Kong/4801/2014	0.5 $TCID_{50}$/mL	100%
		Hong Kong/2671/2019	0.5 $TCID_{50}$/mL	100%
		Switzerland/9715293/2013	0.5 $TCID_{50}$/mL	100%
		Kansas/14/2017 (H3N2)	8 $TCID_{50}$/mL	100%
		Texas/50/2012 (H3N2)	4 $TCID_{50}$/mL	100%
		Wisconsin/15/2009 (H3N2)	0.5 $TCID_{50}$/mL	95.5%
	H5N1 - H5N3	chicken/Vietnam/NCVD-016/2008(H5N1)-PR8-IDCDC-RG12	1:50,000*	100%
		Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	1;100,000*	100%
		Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	1:10,000*	100%
		Duck/Pennsylvania/10218/84 (H5N2)	2.55 pg/mL	100%
		pheasant/New Jersey/1355/1998(H5N2)-PR8-IBCDC-4	1:50,000*	100%
		Duck/Singapore/645/97 (H5N3) V-331-0E5-271	24.8 pg/mL	100%
	H7N2, H7N7, H7N9	A/turkey/Virginia/4529/2002 (H7N2) x PR8-IBCDC-5	1:100,000*	95.5%
		A/mallard/Netherlands/12/2000(H7N7)/PR8-IBCDC-1, genomic RNA	1:100,000*	100%
		A/Anhui/1/2013 (H7N9)	1:100,000*	100%
	H10N7	A/Chick/Germany/N/49 (H10N7)	68 pg/mL	100%
Flu B	Victoria	Brisbane/60/2008	1 $TCID_{50}$/mL	100%
	Victoria	Malaysia/2506/2004	3 $TCID_{50}$/mL	100%
	Yamagata	Phuket/3703/2013	0.5 $TCID_{50}$/mL	95.2%
	N/A	Virginia/ATCC5/2012	0.02 pfu/mL	100%
	Victoria	Washington/02/2019	5 $TCID_{50}$/mL	100.0%
	Yamagata	Wisconsin/1/2010	0.05 $CEID_{50}$/mL	95.5%

Target	Strain		Concentration	% Pos
RSV	RSV A	A (long)	2 pfu/mL	95.5%
		A2001/3-12	8 $TCID_{50}$/mL	95.5%
		A2001/2-20	8 $TCID_{50}$/mL	100%
	RSV B	B, 9320	0.1 pfu/mL	100%
		B1	4 $TCID_{50}$/mL	100%
SARS-CoV-2		USA-IL1/2020	250 cp/mL	95.5%
		USA-AZ1/2020	250 cp/mL	100%
		USA-CA3/2020	250 cp/mL	100%
		Hong Kong/VM20001061/2020	250 cp/mL	100%

The reactivity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay in detection of different clinical isolates of SARS-CoV-2 was demonstrated by performing an *in silico* analysis with the primers and probes of the assay against all the sequences available in GenBank (as of August 12, 2020) using web-based NCBI Basic Local Alignment Search Tool (BLAST). The results show that the primers and probe for SARS-CoV-2 have 100% homology with over 98% of the sequences. Overall, the primers and probe have >95% homology to all sequences analyzed.

Lot-to-Lot Reproducibility

Lot-to-Lot Reproducibility for the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was verified by retrospective analysis of the data generated from qualification tests performed by three operators on three NeuMoDx Systems on three non-consecutive days for three lots of NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test strips manufactured under GMP. Universal Viral Transport medium (UVT) was spiked with 2.0 $TCID_{50}$/mL of a representative strain of Flu A and Flu B, and RSV in addition to SARS-CoV-2 genomic RNA spiked in at 500 copies/mL. The standard deviation for Ct values within and across three lots of NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay test strips was ≤ 1.1 with coefficients of variation (CV) ≤ 3.5% for all targets demonstrating excellent reproducibility, *Table 9*.

Table 9. Reproducibility of Three Lots of NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strips

	Flu A 2.0 $TCID_{50}$/mL			Flu B 2.0 $TCID_{50}$/mL			SARS-CoV-2 (500 copies/mL)			RSV 2.0 $TCID_{50}$/mL			Sample Process Control 2 (SPC2)		
Lot #	C_t Avg	C_t SD	%CV	C_t Avg	C_t SD	%CV	C_t Avg	C_t SD	%CV	C_t Avg	C_t SD	%CV	C_t Avg	C_t SD	%CV
10499X	32.74	0.56	1.7%	32.46	1.10	3.4%	32.35	1.02	3.2%	30.95	0.92	3.0%	26.21	0.43	1.6%
10508X	31.73	0.57	1.8%	32.11	0.56	1.8%	32.70	0.48	1.5%	31.02	0.37	1.2%	25.88	0.73	2.8%
10519X	32.61	0.41	1.3%	32.38	0.27	0.8%	32.71	0.73	2.2%	31.03	0.23	0.7%	26.27	0.29	1.1%
Across Three Lots	32.35	0.69	2.1%	32.31	0.74	2.3%	32.59	0.78	2.4%	31.00	0.58	1.9%	26.12	0.54	2.1%

Clinical Performance

Clinical performance characteristics of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay were determined using an internal retrospective method comparison study using residual nasopharyngeal (NP) swab specimens sourced from two geographically diverse clinical laboratory locations.

Residual NP swab specimens from symptomatic patients were de-identified and given a unique ID number by clinical laboratories, establishing a confidential list linking the patient ID to the de-identified specimens tested for study purposes. Of the 215 individual NP swab specimens tested with both the Direct **and** Pretreated Workflows (total of 439 valid results generated), 30 specimens were identified as Flu A positive, 30 were identified as Flu B positive, 30 were identified as RSV A/B (undifferentiated) positive, and 30 specimens were identified as SARS-CoV-2 positive by the clinical laboratories. Additionally, 50 individual specimens were identified as negative for Flu A, Flu B, and RSV targets and another 50 individual specimens were identified as SARS-CoV-2 negative by the clinical laboratories. The test status of these samples was withheld from the operator to implement a "single blind study". Each specimen was analyzed for each target for each Workflow used for specimen testing. Results reported from the specific FDA- and CE-cleared, legally marketed molecular devices utilized by the laboratories for standard of care testing were used to perform the method comparison analysis.

Results of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay provided a Clinical Sensitivity and Clinical Specificity of 100% for both workflows for the Flu A target (*Table 10A*). Results for the Flu B target provided a Clinical Sensitivity and Clinical Specificity of 96.7% and 98%, respectively, for both workflows (*Table 10B*). Results for the RSV (undifferentiated) target provided a Clinical Sensitivity of 100% for both workflows while the Clinical Specificity was determined to be 98% for the Direct Workflow and 100% for the Pretreated Workflow (*Table 10C*). Results for the SARS-CoV-2 target provided a Clinical Sensitivity of 100% and Clinical Specificity of 98% for both workflows (*Table 10D*). The lower and upper limits of the 95% Confidence Intervals are presented in *Tables 10A, 10B, 10C,* and *10D* below and were calculated using the Wilson procedure with continuity correction.

Table 10A. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of **Flu A**
(a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu A		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	29	0	29
	NEG	0	50	50
	Total	29	50	79
Clinical Sensitivity (Flu A) = 100% (85.4% – 100%)				
Clinical Specificity (Flu A) = 100% (91.1% – 100%)				

(b) Pretreated Workflow

Flu A		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	0	30
	NEG	0	50	50
	Total	30	50	80
Clinical Sensitivity (Flu A) = 100% (85.9% – 100%)				
Clinical Specificity (Flu A) = 100% (91.1% – 100%)				

Table 10B. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of **Flu B**
(a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

Flu B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	29	1	30
	NEG	1	49	50
	Total	30	50	80
Clinical Sensitivity (Flu B) = 96.7% (80.9% – 99.8%)				
Clinical Specificity (Flu B) = 98.0% (88.0% – 99.9%)				

(b) Pretreated Workflow

Flu B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	29	1	30
	NEG	1	49	50
	Total	30	50	80
Clinical Sensitivity (Flu B) = 96.7% (80.9% – 99.8%)				
Clinical Specificity (Flu B) = 98.0% (88.0% – 99.9%)				

Table 10C. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of **RSV A/B** by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

RSV A/B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	1	31
	NEG	0	49	49
	Total	30	50	80
Clinical Sensitivity (RSV A/B) = 100% (85.9% – 100%)				
Clinical Specificity (RSV A/B) = 98.0% (87.9% – 99.9%)				

(b) Pretreated Workflow

RSV A/B		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	0	30
	NEG	0	50	50
	Total	30	50	80
Clinical Sensitivity (RSV A/B) = 100% (85.9% – 100%)				
Clinical Specificity (RSV A/B) = 100% (91.1% – 100%)				

Table 10D. Clinical Performance Summary – NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Test Strip: Detection of **SARS-CoV-2** by (a) Direct Workflow, and (b) Pretreated Workflow

(a) Direct Workflow

SARS-CoV-2		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	1	31
	NEG	0	49	49
	Total	30	50	80
Clinical Sensitivity (SARS-CoV-2) = 100% (85.9% – 100%)				
Clinical Specificity (SARS-CoV-2) = 98.0% (87.9% – 99.9%)				

(b) Pretreated Workflow

SARS-CoV-2		FDA / CE Cleared Reference Test Result		
		POS	NEG	Total
NeuMoDx Flu A-B/RSV/SARS-CoV-2	POS	30	1	31
	NEG	0	49	49
	Total	30	50	80
Clinical Sensitivity (SARS-CoV-2) = 100% (85.9% – 100%)				
Clinical Specificity (SARS-CoV-2) = 98.0% (87.9% – 99.9%)				

Analytical Specificity and Cross-Reactivity

The analytical specificity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage assay was evaluated by testing a panel of 47 organisms, consisting of 22 viral, 24 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in the respiratory tract. Bacteria and yeast were tested at concentrations of ~6E6 CFU/mL or IFU/mL, except where noted. Viruses were tested at concentrations of 1E5 to 1E6 TCID50/mL or copy/mL, except where noted. Analytical specificity of the NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage assay was 100% for Flu A, Flu B, RSV A, RSV B and SARS-CoV-2.

Table 11. Analytical Specificity Results

Organism	Concentration	Flu A	Flu B	RSV A	RSV B	SARS-CoV-2
Adenovirus Type 1	1E6 $TCID_{50}$/mL	-	-	-	-	-
Adenovirus Type 7	1E6 $TCID_{50}$/mL	-	-	-	-	-
Bordetella pertussis I176	10 ng/mL	-	-	-	-	-
Candida albicans	6E6 CFU/mL	-	-	-	-	-
Chlamydia pneumoniae	6E6 IFU/mL	-	-	-	-	-
Corynebacterium xerosis	6E6 CFU/mL	-	-	-	-	-
EBV	1E6 cp/mL	-	-	-	-	-
Escherichia coli	6E6 CFU/mL	-	-	-	-	-
Hemophilus influenzae	6E6 CFU/mL	-	-	-	-	-
HHV 6A	1E6 cp/mL	-	-	-	-	-
HHV 7	1E6 cp/mL	-	-	-	-	-
HHV8	1E6 cp/mL	-	-	-	-	-
HSV1	1E6 cp/mL	-	-	-	-	-
HSV2	1E6 cp/mL	-	-	-	-	-
Human Coronavirus 229E	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human coronavirus HKU1	1E6 cp/mL	-	-	-	-	-
Human coronavirus NL63	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Coronavirus OC43	5E3 $TCID_{50}$/mL	-	-	-	-	-
Human Enterovirus 68	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Metapneumovirus	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 1	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 2	1E5 $TCID_{50}$/mL	-	-	-	-	-
Human Parainfluenza Type 3	1E6 $TCID_{50}$/mL	-	-	-	-	-
Human Rhinovirus Type 1A	1E5 $TCID_{50}$/mL	-	-	-	-	-
Lactobacillus acidophilus	6E6 CFU/mL	-	-	-	-	-
Lactobacillus brevis	6E6 CFU/mL	-	-	-	-	-
Lactobacillus jensonii	6E6 CFU/mL	-	-	-	-	-
Lactobacillus lactis	6E6 CFU/mL	-	-	-	-	-
Legionella pneumophila	6E6 CFU/mL	-	-	-	-	-
Measles	1E5 $TCID_{50}$/mL	-	-	-	-	-
MERS-coronavirus EMC/2012	0.5 ng/mL	-	-	-	-	-
Moraxella catarrhalis	6E6 CFU/mL	-	-	-	-	-
Mumps Virus	1E5 $TCID_{50}$/mL	-	-	-	-	-
Mycobacterium tuberculosis	10 ng/mL	-	-	-	-	-
Mycoplasma pneumoniae	6E6 CFU/mL	-	-	-	-	-
Neisseria gonorrhoeae	6E6 CFU/mL	-	-	-	-	-
Neisseria meningitidis Sero A	6E6 CFU/mL	-	-	-	-	-
Neisseria meningitidis Sero B	6E6 CFU/mL	-	-	-	-	-
Neisseria meningitidis Sero C	6E6 CFU/mL	-	-	-	-	-
Neisseria meningitidis Sero D	6E6 CFU/mL	-	-	-	-	-
Pseudomonas aeruginosa	6E6 CFU/mL	-	-	-	-	-
SARS-coronavirus	1E6 pfu/mL	-	-	-	-	-
Staphylococcus aureus	1E6 CFU/mL	-	-	-	-	-
Staphylococcus epidermidis	6E6 CFU/mL	-	-	-	-	-
Streptococcus pneumonia	6E6 CFU/mL	-	-	-	-	-
Streptococcus pyogenes	6E6 CFU/mL	-	-	-	-	-
Streptococcus salivarius	6E6 CFU/mL	-	-	-	-	-
Flu A (Michigan/272/2017 pdm09 (H1N1)	3x LoD	+	-	-	-	-
Flu B, Florida/78/2015 (Yamagata)	3x LoD	-	+	-	-	-
RSV A, A2	3x LoD	-	-	+	-	-
RSV B (WV/14617/85)	3x LoD	-	-	-	+	-
SARS-CoV-2, USA-WA1/2020	3x LoD	-	-	-	-	+
Negative Control (No Pathogens)	N/A	-	-	-	-	-

Interfering Substances – Commensal Organisms
The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was tested for interference in the presence of non-target organisms (potentially present in the upper respiratory tract) by evaluating the assay performance at low levels (~3X LoD) of Flu A, Flu B, RSV A, RSV B, and SARS-CoV-2 in the presence of high concentrations of the organisms listed in *Table 11*, above. No interference on the detection of any target was observed with any of the commensal organisms.

Interfering Substances – Endogenous/Exogenous
The NeuMoDx Flu A-B/RSV/SARS-CoV-2 Vantage Assay was evaluated for susceptibility to interference caused by substances potentially associated with the collection of nasopharyngeal swab specimens. Residual clinical negative NP swab specimens were individually spiked with Flu A, Flu B, RSV A, RSV B, or SARS-CoV-2 at 3X LoD and processed in the presence and absence of the agents shown in *Table 12*. None of the substances included in the testing had an adverse effect on the assay performance for any of the targets.

Table 12. Substances Tested for Interference

	Substance	Description/Active Ingredient	Concentration*
Exogenous	Neo-Synephrine	Phenylephrine	15% v/v
	Afrin Nasal Spray	Oxymetazoline	15% w/v
	Saline Nasal Spray	Sodium chloride with preservatives	15% v/v
	Zicam Nasal Spray	Luffa operculata, Galphimia glauca, Histaminum hydrochloricum, Sulfur	15% v/v
	Nasal Corticosteroid – Flonase	Fluticasone	5% v/v
	Nasal Corticosteroid – Rhinocort	Budesonide	5% v/v
	Nasal Corticosteroid – Nasacort	Triamcinolone	5% v/v
	Nasal Corticosteroid – Dexamethasone	Dexamethasone	10 mg/mL
	Nasal Corticosteroid – Mometasone	Mometasone	10 mg/mL
	Nasal Corticosteroid – Beclomethasone	Beclomethasone	10 mg/mL
	Chloraseptic Throat Lozenge	Benzocaine, Menthol	2 mg/mL
	Antibiotic, nasal ointment	Mupirocin	10 mg/mL
	Relenza Antiviral Drug	Zanamivir	7.5 mg/mL
	Tamiflu Antiviral Drug	Oseltamivir	25 mg/mL
	Antibiotic, systemic	Tobramycin	1.5 mg/mL
Endogenous	Mucin	Purified Mucin Protein	2.5% w/v
	Human Blood	Blood	2% v/v

*Note: Concentrations shown are those used to saturate swabs before dosing contrived positive clinical samples with interfering substance. They are therefore representative of the level at the site of swab collection that can be tolerated.

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SYMBOL KEY

℞ only	Prescription use only		Temperature limit
	Manufacturer		Do not re-use
IVD	*In vitro* diagnostic medical device	Σ	Contains sufficient for <*n*> tests
EC REP	Authorized representative in the European Community		Consult instructions for use
REF	Catalog number		Caution
LOT	Batch code		Biological risks
	Use-by date	CE	CE Mark

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Revision History

Revision	Date	Summary of Changes
A.1	2020-08	Initial Draft
A.2	2020-09	Added mention of most concentrated common ingredient (21.1% Tris-HCl and 8.4% dNTP) to Materials Provided table. Added Limitation # 9 added (The NeuMoDx™ Flu A-B/RSV/SARS-CoV2 Vantage Test Strip may contain inactive ingredients that may influence the measurement.) Removed biohazard symbol from Specimen section. Correction of minor typos and standardization of Table references in italics. Emergo Response (E0028-R1)
A	2020-09	Same as A.2; provided to Emergo (E0028)
B.1	2020-10	Removal of nasal swab; changed on-board specimen time from 24 hrs to 8 hrs; removed mention of freezing samples for prolonged storage; addition of Analytical Reactivity and Inclusivity and Analytical Specificity and Cross-Reactivity sections; added number of days and operators to Repro Section; correction to Flu B Sensitivity and Specificity in 10B tables.
B.2	2020-10	Removed default setting statement following workflow options presented in Step 1 of NeuMoDx System Operation section (pg 5) due to having 2 ADFs; included “Vantage” in the name of the assay where it was missing; updated Table 10C with 5 more RSV samples and corrected the 95% CI in Table 10D for SARS-CoV-2; corrected the total number of valid results in Clinical Performance section from 429 to 439. Added “3x LoD” for target organisms in and “N/A” for negative control at the bottom of Table 11.
C	2023-07	Updated web reference links to QIAGEN web pages, Safety Data Sheets, technical support, and vigilance supporting Updated Emergo Europe B.V address